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Review

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Innate immune defences in the human endometrium

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Abstract

The human endometrium is an important site of innate immune defence, giving protection against uterine infection. Such protection is critical to successful implantation and pregnancy. Infection is a major cause of preterm birth and can also cause infertility and ectopic pregnancy. Natural anti-microbial peptides are key mediators of the innate immune system. These peptides, between them, have anti-bacterial, anti-fungal and anti-viral activity and are expressed at epithelial surfaces throughout the female genital tract. Two families of natural anti-microbials, the defensins and the whey acidic protein (WAP) motif proteins, appear to be prominent in endometrium. The human endometrial epithelium expresses beta-defensins 1–4 and the WAP motif protein, secretory leukocyte protease inhibitor. Each beta-defensin has a different expression profile in relation to the stage of the menstrual cycle, providing potential protection throughout the cycle. Secretory leukocyte protease inhibitor is expressed during the secretory phase of the cycle and has a range of possible roles including anti-protease and anti-microbial activity as well as having effects on epithelial cell growth. The leukocyte populations in the endometrium are also a source of anti-microbial production. Neutrophils are a particularly rich source of alpha-defensins, lactoferrin, lysozyme and the WAP motif protein, elafin. The presence of neutrophils during menstruation will enhance anti-microbial protection at a time when the epithelial barrier is disrupted. Several other anti-microbials including the natural killer cell product, granulysin, are likely to have a role in endometrium. The sequential production of natural anti-microbial peptides by the endometrium throughout the menstrual cycle and at other sites in the female genital tract will offer protection from many pathogens, including those that are sexually transmitted.

Introduction

The key role of the human endometrium is to orchestrate the events that lead to fertilization, implantation and pregnancy. The prevention of uterine infection is crucial to successful human reproduction and although the endometrium must function as an efficient mucosal barrier the passage of spermatozoa has to be accommodated. The innate immune system in endometrium, as elsewhere, must protect against infection while also signalling

the presence of a pathogen to the acquired immune system in the event that infection does occur. Natural anti-microbials are gene-encoded peptides that are key mediators of the innate immune system and the primary focus of this review is to describe their expression in the human endometrium

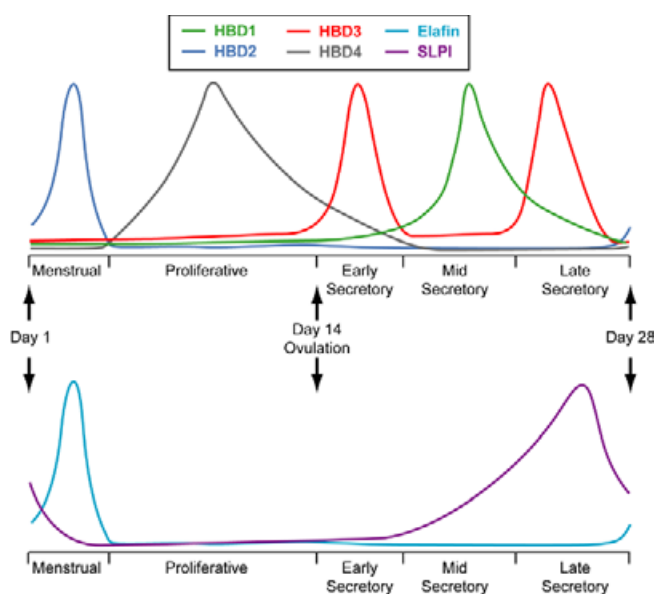


Figure 1
Representation of peak expression of the β -defensins, HBD1-4, and the WAP proteins, SLPI and elafin, in endometrium throughout the menstrual cycle. HBD1, HBD3 and SLPI are maximally expressed during the secretory phase. In contrast, expression of HBD4 is highest during the proliferative phase while HBD2 and elafin expression peaks during menstruation.

The menstrual cycle

The human endometrium undergoes characteristic cyclical changes in response to the steroid hormones, oestradiol and progesterone. These have been described in detail by Noyes et al [1]. In the first half of the menstrual cycle (proliferative phase, days 4–13), when oestradiol concentrations are increasing due to the development of a follicle, the endometrium undergoes proliferation. Ovulation occurs on day 14 of the cycle, the corpus luteum forms and progesterone concentrations increase thereafter. Under the influence of progesterone endometrial growth ceases and differentiation occurs in preparation for implantation and pregnancy. The secretory function of the endometrial glands increases with maximal secretion in the mid secretory phase (days 19–23) around day 20. In the late secretory phase (days 24–28) spiral arteriole differentiation occurs and the stromal cells in immediate proximity to these vessels differentiate (decidualize). The nuclei of these cells become enlarged and the cytoplasmic volume increases. These changes also occur in the stromal cells below the surface epithelium and, if pregnancy occurs, the entire endometrium decidualizes. In the absence of implantation menstruation occurs resulting in tissue breakdown and repair allowing regeneration of the

endometrium for the subsequent cycle. There are also cyclical changes to the leukocyte populations present in endometrium and these are described below.

Natural anti-microbials

The two major families of natural anti-microbials that will be discussed in relation to endometrium are (i). defensins and (ii). whey acidic protein (WAP) motif containing proteins. Studies in other mucosal systems such as the respiratory tract [2] strongly suggest that other anti-microbials will also be active within the uterus.

Defensins

Defensins are a family of small cationic proteins that have been shown to confer the host with anti-bacterial, anti-fungal and anti-viral protection. Defensins have six cysteine residues that form three disulphide bonds and they are divided into two main groups on the basis of the position of these bonds: α -defensins are found in neutrophils (human neutrophil peptides (HNP) 1-4) and at epithelial sites (human defensin (HD) 5 and 6) while the β -defensins (human β defensins (HBD)) are found mainly at epithelial surfaces e.g. gut, lung [3,4]. It has recently been reported that there are 28 defensin-like sequences in the human genome [5] but currently only HBD1-6 have been characterized [6-11]. The β -defensins further subdivide into those that are constitutively expressed (e.g. HBD1) and those that are induced upon challenge with inflammatory or pathogen-derived stimuli [9,10,12-14]. In addition to their role as anti-microbials, the β -defensins are chemoattractants allowing an interaction between the innate and the acquired immune systems [8,9,15]. The expression, regulation and role of the β -defensins at non-reproductive sites has been reviewed elsewhere [16-18].

WAP motif containing proteins

Proteins containing the WAP motif have a 50 amino acid domain, which forms a tight core containing four disulphide bonds [19]. This family includes secretory leukocyte protease inhibitor (SLPI), elafin, eppin and HE4 [20]. SLPI and elafin are the most widely studied WAP proteins. These two molecules share 40% homology, with eight identical cysteines and five identical prolines, and both are expressed at mucosal surfaces where they have anti-protease and anti-microbial actions [21-26]. Both proteins inhibit serine proteases with SLPI inhibiting several proteases including neutrophil elastase and cathepsin G [27] while elafin regulates only neutrophil elastase and proteinase 3 [28-30]. These actions combined with their anti-microbial activity allow SLPI and elafin to protect epithelial surfaces during infection and inflammation. The similarity between SLPI, elafin and other proteins containing the WAP motif suggests that they may also

have anti-protease and anti-microbial actions at epithelial surfaces [20].

Natural anti-microbial expression by the human endometrium

There are two main sites of natural anti-microbial expression in the endometrium: the epithelium and the leukocyte populations that are an important component of the cell types constituting the endometrium.

Natural anti-microbial expression by the endometrial epithelium

As at other mucosal surfaces the epithelial cell mediates the initial response of the endometrium to infection [31]. In 1997 and 1998 the first reports of defensin expression in the human endometrium were published. These described the expression of the α -defensin, HD5, and the β -defensin, HBD1, in the endometrial epithelium [32-34]. Subsequently, messenger RNA (mRNA) for HBD1-4 has been detected in endometrium and it has been shown that each defensin has a unique temporal expression profile [35,36]. HBD1 and 3 are expressed at highest levels during the secretory phase [35,36]. A similar pattern of expression has been reported for HD5 [33]. In contrast, HBD2 mRNA expression shows a dramatic peak during menstruation while HBD4 is expressed mainly in the proliferative phase [35,36]. These data are summarised in Figure 1. The reasons for these differential patterns of expression have not been determined and may relate to differences in anti-microbial activity of members of the defensin family or to their other activities such as chemotaxis. Acquisition of sexually transmitted infections is reported to be influenced by oestradiol and progesterone [37] and changes to the expression of innate immune molecules such as defensins may contribute to this. As in other epithelial cells, expression of both HBD2 and 3 in endometrial epithelial cells can be upregulated in response to inflammatory stimuli suggesting that these defensins will be induced in response to infection [36,38].

The WAP protein, SLPI, has also been detected in the endometrial epithelium with peak expression in the mid-late secretory phase (Figure 2a) [39]. This protein may have important actions during the window of implantation. SLPI has several anti-inflammatory actions including inhibition of the proinflammatory transcription factor, nuclear factor κ B [40], and this combined with its anti-protease activity may prevent an excessive inflammatory response from occurring in the uterus at the time of implantation and during early pregnancy. In addition, SLPI has recently been suggested to have an effect on epithelial growth. Proepithelin (PEPI) is an epithelial growth factor that can be converted to a growth inhibitor, epithelin (EPI), by elastase. SLPI can form a complex with PEPI preventing its cleavage by elastase and hence, promote

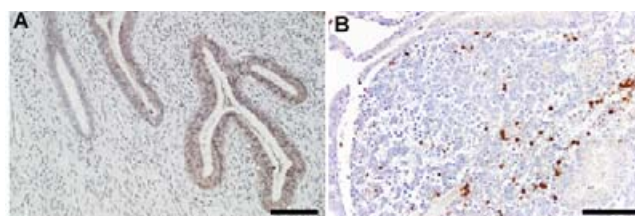


Figure 2

Immunohistochemical localization of the WAP proteins, SLPI and elafin, in human endometrium. **(A)**, SLPI immunolocalization in endometrium from the mid secretory phase. Immunoreactivity is present in the glandular epithelium and secretions. **(B)**, Elafin immunolocalization in menstrual endometrium. Immunoreactivity is present in neutrophils infiltrating the endometrial stroma. Scale bars = 100 μ m.

epithelial growth [41]. In vitro studies using the Ishikawa epithelial cell line (derived from an endometrial adenocarcinoma) have shown that SLPI can also increase expression of the cyclin D1 gene while inhibiting expression of anti-proliferative genes such as insulin-like growth factor binding protein-3 and lysyl oxidase [42]. The anti-microbial actions of SLPI are likely to be particularly important in the protection of the uterus from infection during implantation and early pregnancy. Indeed, it has been shown that the antibacterial activity of apical secretions from polarized endometrial epithelial cells can be reduced by incubation with an anti-SLPI antibody confirming that SLPI contributes to the antibacterial defences at the epithelial surface at least in vitro [43]. SLPI concentrations and the ratio of SLPI to elastase have also been found to increase in the peritoneal fluid of women suffering from endometriosis. Local peritoneal inflammation is one of the factors thought to be responsible for the symptoms of this disease and it has been suggested that SLPI concentrations are increased in response to this and that the anti-inflammatory and anti-protease actions of SLPI may limit the inflammatory process [44].

It is likely that the endometrial epithelium will also express additional natural anti-microbials. For example, the iron-binding protein lactoferrin is expressed by the endometrial epithelium [45]. Lactoferrin has anti-microbial actions [46] and has been shown to be oestrogen regulated with maximal expression in the proliferative phase [47]. Lactoferrin has been shown to act in synergy with SLPI [48] and it is likely that interactions between natural anti-microbials in endometrium will also be important.

Natural anti-microbial expression by endometrial leukocytes

Leukocyte populations are an important component of the endometrium, constituting 8.2% of the stroma during the proliferative phase with this figure increasing to 31.7% in first trimester decidua [49]. The presence of several leukocyte populations in human endometrium is cycle dependent. The populations of neutrophils, uterine NK cells (uNK), macrophages and mast cells are relevant to endometrial anti-microbial expression. uNK cells are present in endometrium in the mid-late secretory phase of the menstrual cycle and are the major leukocyte population in first trimester decidua accounting for 70% of the leukocytes present at this time [50]. They are believed to have a role in implantation, decidualization and placenta-tion [51]. uNK cells differ from the main population of NK cells in peripheral blood as they are CD56^{bright} [51]. The presence of these cells in endometrium is dependent on progesterone although this may be an indirect action, as uNK cells do not express the nuclear progesterone receptor [52]. These cells express receptors for the glucocorticoid receptor and oestrogen receptor β suggesting that they do respond to these steroid hormones [53]. Macrophages are present throughout the cycle with a small increase in number premenstrually [50,54]. There are also increased numbers present in early pregnancy with a high concentration found at the implantation site [55]. Immediately prior to menstruation there is an influx of neutrophils into the endometrium [56]. Leukocytes are involved in the tissue breakdown and repair that occurs at this time [57]. Infiltration of endometrium with both neutrophils and macrophages around the time of menstruation is coincident with falling progesterone concentrations. The effects of progesterone withdrawal on these leukocyte populations is believed to be mediated by increased expression of inflammatory mediators such as interleukin-8 and monocyte chemoattractant protein-1 in the endometrium premenstrually [58-60]. Mast cells are also present throughout the menstrual cycle, although in smaller numbers, and are activated at menstruation [61].

Neutrophils are a rich source of natural anti-microbial molecules (Figure 3). The infiltration of the endometrium by neutrophils during menstruation will enhance natural anti-microbial protection at a time when the epithelial barrier is disrupted. In addition, neutrophils will rapidly infiltrate during infection, again increasing the innate defences at an appropriate time. Peripheral blood neutrophils have been shown to express α -defensins, the cathelicidin, human cationic anti-microbial peptide 18 (hCAP-18), lactoferrin and lysozyme [62-64]. It is likely that endometrial neutrophils will also express these molecules although this has not yet been documented. Moreover, it is possible that the cytokine environment in the uterus may, at certain stages of the menstrual cycle, mod-

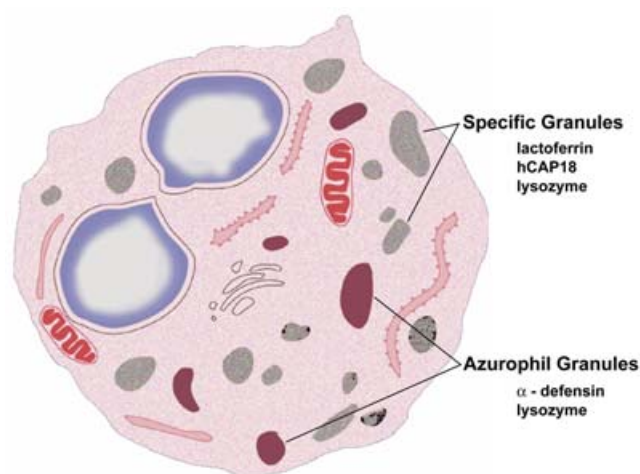


Figure 3

Natural anti-microbial expression in the human neutrophil. Neutrophils contain two types of granules that contain natural anti-microbials. The azurophil granules contain α -defensins while specific granules contain lactoferrin and the cathelicidin, hCAP-18. Lysozyme is present in both granule types.

ulate neutrophil granule release. The WAP protein, elafin, has been detected in endometrial neutrophils (Figure 2b) [65]. Elafin mRNA and protein expression rises dramatically around the time of menstruation and immunohistochemical studies have determined that the cellular source of endometrial elafin is the neutrophil. The differing expression profiles of the two WAP proteins, SLPI and elafin (Figures 1 and 2), in endometrium suggest that they have independent roles. The anti-protease actions of elafin may aid tissue repair during menstruation while the protein's anti-microbial activity will increase innate immune defences at this time.

Granulysin is an anti-microbial molecule that is stored in the granules of cytolytic T cells and peripheral blood NK cells [66]. Granulysin mRNA has been found to be maximally expressed in endometrium from the late secretory phase suggesting that the uNK cell is also a source of this anti-microbial [35]. Expression of granulysin during the late secretory phase may provide additional protection around the time of implantation or in its absence, menstruation.

It is also likely that endometrial macrophages and mast cells will express natural anti-microbial peptides. There are reports that HBD1 and 2 are expressed by blood monocytes and alveolar macrophages [67] and that hCAP-18 is expressed by human skin mast cells [68].

Table 1: Natural anti-microbial expression in the female reproductive tract.

Site of expression	Anti-microbial	Reference
Vagina	HD5	[33]
	HBD1 ¹	[34]
	Elafin ²	[81]
	hCAP-18	[82]
Cervix	HD5	[32,33]
	HBD1 ¹	[34]
	Elafin ²	[81]
	SLPI ²	[83,84]
Fallopian tube	hCAP-18	[82]
	HD5 (variable)	[33]
	HBD1 ¹	[34]
	SLPI ²	[85]
First trimester decidua	SLPI ²	[39]
Amnion/Amniotic fluid	SLPI ²	[86,87]
Term decidua	Lactoferrin	[88]
	SLPI ²	[86]
	Lactoferrin	[88]
Chorion	HD5	[32]
Placenta	HNP1 or 3	[89]
	HBD1 ¹	[90]
	HBD3 ¹	[91]

¹chemoattractant role ²anti-protease activity

However, localization of these peptides to endometrial leukocytes has not yet been described.

Importance of natural anti-microbial expression to reproductive health

Mucosal surfaces of the endometrium do not act in isolation to protect against infection. Anti-microbial peptides are expressed at sites throughout the female reproductive tract (Table 1) in order to protect the upper genital tract and, in pregnancy, the developing fetus, from infection. The consequences of such an infection include infertility, ectopic pregnancy and preterm birth [69,70]. However, little is known about the effects of contraceptive use on natural anti-microbial expression.

The combined oral contraceptive pill (COCP) can alter the susceptibility of users to pelvic infection. For example, the prevalence of infection of the cervix with Chlamydia is increased [71] while symptomatic Chlamydial infection of the upper genital tract is decreased in COCP users compared to non-users [72]. Oral contraceptive users are also at higher risk of gonococcal infections [73]. These differences are likely to be due to several factors including changes to the viscosity of cervical mucus, alterations to cell mediated immunity and decreased menstrual blood flow [37,74]. Changes to innate immune factors are also likely to be important. Expression of the β -defensins, HBD1-4, is suppressed in COCP users compared to peak expression of these defensins in non-users suggesting that

innate immune defences are altered [35,36]. This may have important consequences and it is clear that future contraceptive design should ascertain the effects on anti-microbial molecules. New contraceptives should not have a deleterious effect on the innate immune system and ideally, might enhance its actions.

Uterine infection is a major contributory factor in preterm births [70]. There are studies that suggest that the expression of anti-microbial molecules is upregulated in response to this situation. Concentrations of lactoferrin increase in amniotic fluid in patients with intra-amniotic infections, whether in preterm or term labour [75]. In addition, levels of the α -defensins, HNP1-3, are increased 4–24 fold in patients in preterm labour suffering from infection [76]. It is not yet clear whether or not any defects in expression of anti-microbial molecules exist that may increase susceptibility to uterine infections during pregnancy. The role of anti-microbials in the acquisition of sexually transmitted infections has also not been fully established although one study reports a decrease in SLPI concentrations in vaginal fluid from women suffering from lower genital tract infections [77]. In contrast, there are reports to suggest that high concentrations of SLPI may be beneficial in the limitation of spread of HIV. SLPI has been shown to be the active component of saliva that inhibits infection of monocytes with HIV [78] and there is evidence to suggest that high concentrations of SLPI in vaginal fluid reduce the likelihood that an infant will be

infected with HIV during vaginal delivery [79]. Similarly, high concentrations of SLPI in infant saliva are reported to offer protection against HIV transmission during breast-feeding [80].

Conclusions

The human endometrium, in common with other mucosal surfaces, produces a wide array of natural antimicrobial peptides with the appearance of these peptides appearing to be governed by the stage of the menstrual cycle. These are expressed by both epithelial cells and endometrial leukocytes and have the potential to provide protection throughout the menstrual cycle and in pregnancy. The actions of these molecules may limit the spread of sexually transmitted infections and prevent against the uterine infections that cause preterm birth.

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